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Abstract

Reproductive performance is the most important component of cattle production from the standpoint of economic sustainability of commercial beef enterprises. Heifer Pregnancy (**HPG**) and Stayability (**STAY**) genetic predictions are 2 selection tools published by the Red Angus Association of America (**RAAA**) to assist with improvements in reproductive performance. Given the importance of HPG and STAY to the profitability of commercial beef enterprises, the objective of this study was to identify QTL associated with both HPG and STAY in Red Angus cattle. A genome-wide association study (**GWAS**) was performed using deregressed HPG and STAY EBV, calculated using a single-trait animal model and a 3-generation pedigree with data from the Spring 2015 RAAA National Cattle Evaluation. Each individual animal possessed 74,659 SNP genotypes. Individual animals with a deregressed EBV reliability > 0.05 were merged with the genotype file and marker quality control was performed. Criteria for sifting genotypes consisted of removing those markers where any of the following were found: average call rate less than 0.85, minor allele frequency < 0.01, lack of Hardy–Weinberg equilibrium ($P < 0.0001$), or extreme linkage disequilibrium ($r^2 > 0.99$). These criteria resulted in 2,664 animals with 62,807 SNP available for GWAS. Association studies were performed using a Bayes Cπ model in the BOLT software package. Marker significance was calculated as the posterior probability of inclusion (**PPI**), or the number of instances a specific marker was sampled divided by the total number of samples retained from the Markov chain Monte

Carlo chains. Nine markers, with a PPI ≥ 3% were identified as QTL associated with HPG on BTA 1, 11, 13, 23, and 29. Twelve markers, with a PPI ≥ 75% were identified as QTL associated with STAY on BTA 6, 8, 9, 12, 15, 18, 22, and 23.

Keywords: Heifer Pregnancy, quantitative trait loci, Red Angus, Stayability

Introduction

Reproductive performance is an important factor associated with the economic viability of beef enterprises. For most identified economically relevant traits (Golden et al., 2000), the beef industry has done a relatively thorough job of providing producers with accurate selection tools in the form of EPD that can be used to improve the aggregate breeding value of their herds; however, genetic predictions for reproductive traits represent an exception to this generality. Heifer Pregnancy (**HPG**) and Stayability (**STAY**) are 2 selection tools that can be used for targeted improvement of beef female reproduction that have been available to Red Angus Association of America (**RAAA**) members since the mid- to late 1990s; yet these have some inherent weaknesses.

Reproductive traits like HPG and STAY have several challenges such as a lack of pregnancy data reported to the association, low heritability estimates, and the inherent nature of the trait with the extended length of time to accumulate observations occurring later in the life span of females. These weaknesses have resulted in suboptimal genetic progress for both traits. In addition to these problems, the models used for the evaluation of HPG have remained stagnant since their inception, while STAY on the other hand has received some attention in recent years (e.g., Brigham et al., 2007; Jamrozik et al., 2013). Relatively recently, the availability of DNA information in the form of SNP chips has allowed insight into many other traditional beef cattle traits. Identified QTL associated with both HPG and STAY can be used to help identify causal mutations as well as used to enhance the models that are currently used in national cattle evaluation by adding additional accuracy to the resulting predictions at younger animal ages. Consequently, the objectives of this study were to conduct genome-wide association studies (**GWAS**) to identify chromosomal regions in Red Angus cattle associated with both HPG and STAY.

Materials and methods

The data used in the current study were obtained from an existing database; therefore, the study was not subject to animal care and use committee approval.

Phenotypic Data

Estimated breeding values and corresponding accuracies for both HPG and STAY were calculated from the data sent to Colorado State University for the Spring 2015 National Cattle Evaluation from the RAAA. Heifer Pregnancy is a prediction of a heifer's ability to conceive during her first breeding season, and STAY is a prediction of a female's ability to produce 5 consecutive calves by 6 yr of age. Given the binary nature of both of these traits, EBV were estimated by converting the observations to an underlying normal distribution using a *maximum* a posteriori probit threshold model (Gianola and Foulley, 1983; Harville and Mee, 1984). The models used for the calculation of EBV contained the fixed effects of contemporary group for HPG and STAY as well as breeding age as a linear covariate for HPG. Contemporary groups were formed using guidelines obtained from the RAAA National Cattle Evaluation. The contemporary group for HPG consisted of yearling weight contemporary group in addition to a heifer management code. The STAY contemporary group definition consisted of birth year and breeder of the female and breeder of each of her 5 calves. A total of 29,333 heifers with HPG and 215,920 females with 6-yr STAY observations were used for the estimation of breeding values. A 3-generation pedigree was created for individuals in each of the final data files, resulting in pedigree sizes of 104,100 and 405,640 for HPG and STAY, respectively. Heritability on the underlying scale for HPG was 0.12 and for STAY was 0.10 (Boldt, 2017).

Following the procedures outlined in Garrick et al. (2009), these EBV were deregressed (**DEBV**) and the effects of the parental average EBV and accuracy were removed. Deregressed EBV for each of the traits were weighted using the heritabilities presented above and the assumption that the proportion of genetic variance not explained by the markers was 0.40 (Saatchi et al., 2012). After deregression and removal of parental average EBV and accuracy, animals with DEBV reliability less than 0.05 were removed from the analysis following the recommendations of Zeng (2016). Removal of these individuals with low DEBV reliability, resulted in 83,635 and 328,507 animals with HPG and STAY DEBV, respectively, that were considered for further analyses.

Genotype Data

Genotype data were generated from a separate body of work, which is described by Lee et al. (2017). In summary, a total of 9,776 Red Angus beef cattle were genotyped using 5 different SNP panels: BovineSNP50 v2 (Illumina Inc., San Diego, CA), GGPLD v1, GGPLD v2, GGP-HD, and GGP-HD v2 (Neogen Agrigenomics, Lincoln, NE). These SNP panels were imputed to a medium density SNP panel consisting of approximately 80 K SNP markers that matched the file named GeneSeek Genomic Profiler HD (76,879 SNP)

on SNPchiMp (version 3: http://bioinformatics.tecnoparco.org/SNPchimp/). After further evaluation of the manifest file, it was determined that 74,403 SNP genotypes were available for GWAS. Given the phenotypic data were sent to Colorado State University for the Spring 2015 National Cattle Evaluation (performed in December 2014), a number of animals with genotypes were not in the phenotypic data file. A total of 6,575 individuals in the genotype file were also present in the Spring 2015 National Cattle Evaluation.

Individual animals whose DEBV reliability was greater than 0.05 were merged with the genotype file, and marker quality control was performed. Specifically, genotypes at each locus were filtered according to the following criteria: average call rate lower than 0.85, minor allele frequency less than 0.01, markers not in Hardy–Weinberg equilibrium whose *P*-value was less than 1×10^{-4}, and for SNP in extreme linkage disequilibrium ($r^2 > 0.99$). Any marker having at least one of the previous criteria was removed from the analysis. After these filters, 60,967 SNP genotypes were available for GWAS.

Genotype to phenotype (DEBV proportionally weighted to the resulting reliability) association using the Bayes Cπ model (Habier et al., 2011) was implemented using the BOLT software package (http://www.thetasolutionsllc.com/bolt-software.html) to identify chromosomal regions associated with both HPG and STAY. The value of π, or the fraction of markers with a null effect for HPG or STAY, was set to 0.99. Individual markers were evaluated using 4 parallel Markov chain Monte Carlo (**MCMC**) chains of 70,000 iterations with a 10,000 iteration burn-in for a total of 240,000 samples. Marker estimates and variances were averaged across the 4 chains, and the number of times a specific marker was included in the chain was summed. The number of instances a single marker was included in a chain was divided by the total number of samples and averaged across the 4 chains to obtain the posterior probability of inclusion (**PPI**). Markers were then ordered according to the UMD3.1.1 (Zimin et al., 2009) assembly and marker location vs. PPI was plotted to identify influential chromosomal regions.

Results and discussion

Summary statistics for the raw EBV and corresponding accuracy are presented in **Table 1**. Mean EBV for both HPG and STAY were −0.031 and 0.083, respectively. Accuracy values corresponding to HPG ranged from 0.00 to 0.998 and accuracy values corresponding to STAY ranged from 0 to 0.999. The EBV summarized in Table 1 were deregressed, and then subsequently merged with the genotype data to serve as dependent variables in the GWAS. Summary statistics describing the DEBV used as response variables in the GWAS are summarized in **Table 2**. A total of 567 individuals for HPG and

Table 1. Raw Heifer Pregnancy and Stayability EBV and accuracy summary statistics

		Trait	
	Item	*Heifer Pregnancy*[a]	*Stayability*[a]
	N	104,100	405,640
Average	EBV	−0.031	0.083
	Accuracy[b]	0.604	0.628
SD	EBV	0.098	0.134
	Accuracy[b]	0.126	0.108
Minimum	EBV	−0.687	−0.844
	Accuracy[b]	0	0
Maximum	EBV	0.421	0.871
	Accuracy[b]	0.998	0.999

a. Both Heifer Pregnancy and Stayability predictions are presented as underlying EBV.
b. Accuracy is presented as true accuracy or r_{TI}.

2,664 individuals for STAY possessed both genotype and the DEBV response variable. DEBV reliabilities ranged from 0.00 to 0.995 for HPG and from 0.00 to 0.997 for STAY. The number of HPG observations included in the evaluation of HPG was concerning and results should be interpreted accordingly.

For the HPG genetic evaluation, breeding information was the most sparsely reported information in the RAAA database. Because of this, a majority of the individuals with predictions for HPG have very low accuracies. Contrary to HPG, STAY is another reproductive trait where one would expect lower reported data densities; however, STAY observations were formed for a female based on reported calf weaning weight observations. Since STAY records are formed on the presence or absence of a calf, a greater number of observations are evaluated in those predictions. Policy of the RAAA is that a weaning weight must be reported to the Association for a calf to be registered (RAAA, 2017). Also, because of the nature of the way STAY records are formed, there is a greater representation of the entire RAAA population in the evaluation, whereas HPG observations require breeders to submit

Table 2. Heifer Pregnancy and Stayability deregressed EBV summary statistics for Red Angus cattle with genotype information

	Heifer Pregnancy	*Stayability*
N	567	2,664
Average	0.0045	0.2553
SD	0.2215	0.1170
Minimum	−2.363	−0.823
Maximum	1.040	2.056

additional breeding information. As a result, HPG data submission occurs on a small subset of animals. Additionally, when comparing animals that possess genotype information to those that possess actual HPG measures or to those that are closely related to individuals with HPG records, little overlap between the 2 groups exists, meaning a very small number of individuals possess both pieces of information. This is due in part to the fact that male genotypes are overrepresented when compared to females that possess observations for these traits. Also, for STAY, given the time lag for observations, there are many young, genotyped animals that would not have daughters with records. As such, perhaps targeted data collection and/or genotyping needs to be performed to help fill these voids. It is critical to have large numbers of animals that possess both genotype and phenotype information, therefore some form of genotyping and phenotyping strategy where both pieces of information are collected on females should be implemented.

Results from genome-wide associations in this study were reported for single SNP markers as opposed to marker windows. The reasoning behind this is the recent discovery of a number of assembly problems in the current UMD3.1.1 release. A new bovine reference assembly project (ARS-UDC v1.0) is underway that will likely address many of the issues of the current assembly (Zhou et al., 2015; Medrano, 2017). The new assembly is considered an improvement over the previous assemblies, and this improvement is large enough that Medrano (2017) suggested GWAS reanalysis of previous studies should be conducted. Reported results for single SNP associations obtained from GWAS activities should not change with the new assembly. What may, perhaps change is the designated location of these SNP on a given chromosome. Results reported in this study should not change in terms of the specific SNP identified; however, results are going to be discussed in terms of the individual SNP locations from the UMD3.1.1 annotation and assembly. Additionally, bioinformatic tools provided by the National Animal Genome Research Program (https://www.animalgenome.org) and by the University of California at Santa Cruz genome browser (https://genome.ucsc.edu) were used to identify genes which underlie the described QTL.

Previously, in Brangus females, Fortes et al. (2012) identified individual markers on BTA 2 and 28 with a significant influence on HPG. An additional study with the same data, Peters et al. (2013), identified SNP windows influencing HPG on BTA 2, 4, 8, 10, 13, and 20. We identified SNP on BTA 1, 11, 13, 23, and 29. It is unknown if the discrepancies between the studies are due to breed differences, with Brangus cattle being 3/8 Brahman, the methods employed, or simply a sampling issue relative to animals with available data in each population.

Results from the GWAS for HPG are shown in **Table 3** and the corresponding Manhattan plot is presented in **Fig. 1**. For HPG, 9 markers had a

Table 3. SNP associated with Heifer Pregnancy in Red Angus cattle

dbSNP[a]	*BTA*[b]	*Location (Mb)*	*PPI*[c]	*Gene*	*Gene location*[d]
rs133170162	1	12.24	0.03		
rs43268408	1	131.94	0.04	ARMC8	Intron
rs133044215	11	14.55	0.05	MEMO1	17 kb
rs137181195	11	44.19	0.04	SEPT10	Intron
rs134129467	11	51.19	0.03		
rs134135677	13	30.50	0.03		
rs136869280	23	7.31	0.03	COL11A2	Intron
rs110079780	23	10.94	0.06	FGD2	18.4 kb
rs110367141	29	9.11	0.04	ME3	Intron

a. SNP name from dbSNP.
b. Chromosome.
c. Posterior probability of inclusion in the model.
d. Location of SNP within or distance from any annotated genes.

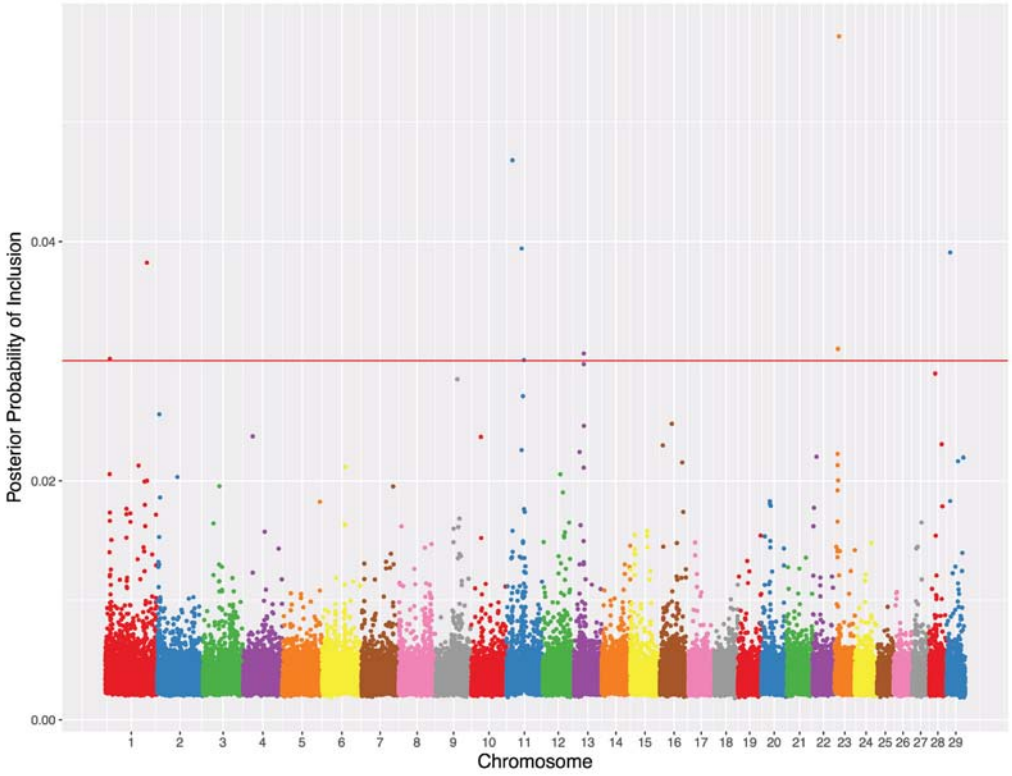

Figure 1. Manhattan plot identifying SNP associated with Heifer Pregnancy in Red Angus cattle. The red horizontal line denotes a 3% PPI.

PPI that ranged from 0.03 to 0.06, and were identified on BTA 1, 11, 13, 23, and 29. The level of inclusion (PPI) associated with markers for HPG was low. The mean PPI for HPG was 0.33% and the maximum PPI for HPG was 6%, meaning out of the 240,000 samples, this marker was sampled 14,440 times. As discussed previously, the lack of HPG phenotypic information on genotyped animals in the RAAA data combined with the low heritability of HPG (h^2 = 0.12) are likely the primary drivers of the lack of identified SNP for HPG.

Of the SNP with the strongest signal for HPG, however, a total of 4 markers rs43268408 (BTA 1), rs137181195 (BTA 11), rs136869280 (BTA 23), and rs110367141 (BTA 29) were located within non-coding regions of mapped genes. Most interestingly though, the marker with the greatest PPI, rs110079780, located at 10.9 Mb on BTA 23, was reported within previously described QTL for calving ease and calving index in Holstein cattle (Sahana et al., 2010). Perhaps the QTL identified for calving ease and calving index are more generally associated with bovine reproduction. However, in order to fully determine if these SNP are truly associated with HPG, additional data are needed.

Results obtained from the STAY evaluation are presented in **Table 4**, with the corresponding Manhattan plot in **Fig. 2**. In this study, we identified 12 markers on 8 chromosomes (BTA 6, 8, 9, 12, 15, 18, 22, 23) whose PPI was greater than 0.75 (Table 4) meaning they were selected for model inclusion in 75% of the MCMC samples. Previous research has shown SNP associated with STAY on BTA 4, 5, 15, and 19 from 222 Nellore–Angus cross bred cattle

Table 4. SNP associated with Stayability in Red Angus cattle

dbSNP[a]	*BTA*[b]	*Location (Mb)*	*PPI*[c]	*Gene*	*Gene location*[d]
rs42446897	6	12.97	0.75	CAMK2D	Intron
rs42330036	6	105.13	0.77	CRMP1	Intron
rs42280921	8	46.91	0.95	KLF9	Intron
rs42491470	9	13.85	0.83		
rs42728110	9	16.98	1.00	HTR1B	31 kb
BTA-30939-no-rs	12	83.54	0.78		
rs110397539	15	58.78	0.95		
rs42216237	15	60.67	0.97		
rs42364231	15	74.86	0.82	ALKBH3	Intron
rs109374253	18	61.16	0.85	NRLP12	13.5 kb
rs43598673	22	55.11	0.84	ATP2B4	Intron
rs10938184	4 23	14.84	0.80		

a. SNP name from dbSNP.
b. Chromosome.
c. Posterior probability of inclusion in the model.
d. Location of SNP within or distance from any annotated genes.

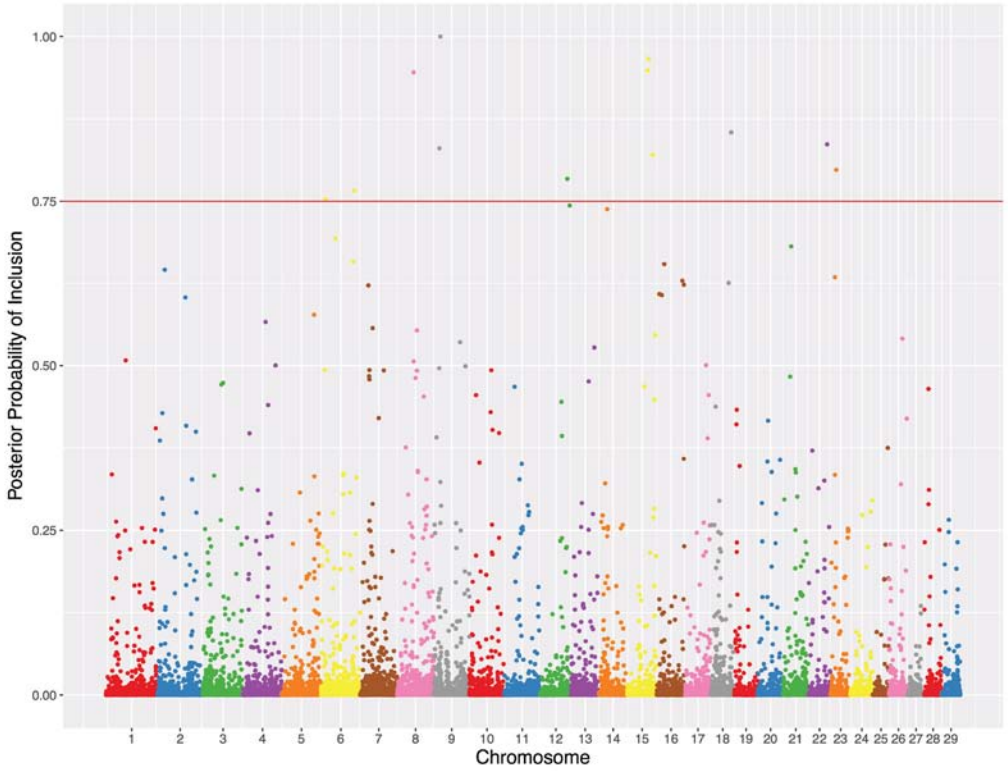

Figure 2. Manhattan plot identifying SNP associated with Stayability in Red Angus cattle. The red horizontal line denotes a 75% PPI.

(Engle et al., 2016) which were found near dairy QTL for calving interval (BTA 5), length of productive life (BTA 15), and fertility (BTA 19). Other than BTA 15, the regions we identified were not reported in the work of Engle and colleagues; however, given their data were from a *Bos indicus*-crossed population, this discordance in identified chromosomal regions was not surprising.

For the trait STAY, 4 SNP whose PPI was ≥95% were identified on BTA 8, 9, and 15. On BTA 8, rs42280921 (95% PPI) was located at 46.91 Mb. This SNP was located within an intron of the gene *Kruppel Like Factor 9* (KLF9). The identified SNP, rs42728110 (100% PPI) was located at 16.98 Mb on BTA 9 was not found to be within any annotated genes. However, expanding the viewed window, the gene *5-Hydrosytraptamine Receptor* 1B (HTR1B) was located 31 kb downstream from this SNP. Two SNP were identified on BTA 15 with a PPI ≥ 95%. First, rs110397539 (95% PPI) was located at 58.8 Mb on BTA 15. This marker was located in a SNP window that explained 1.52% of the genetic variance associated with feed conversion ratio in Nelore cattle (de Oliveria et al., 2014). In that body of work, the authors identified a 1 Mb SNP window located at 58.0 Mb on BTA 9. In that window, the authors reported the candidate gene *Lin-7 Homolog C* (LIN7C), and described the

association with variability in obesity and type-2 diabetes in humans (Ng et al., 2010). Given that STAY is a predictor of lifetime production efficiency, it is sensible to assume that more feed efficient animals would have greater energy reserves for physiological systems such as reproduction resulting in a greater probability of remaining productive until 6 yr of age. The other SNP on BTA 15 whose PPI was ≥95% was rs42216237 (97% PPI) located at 60.67 Mb on BTA. There were no annotated underlying genes identified within this region, although these 2 SNP surround the previously reported QTL.

Five SNP were found to be associated with variability in STAY whose PPI was less than 95% but ≥85%. Marker, rs42364231 (82% PPI) was identified on BTA 15 at 74.86 Mb. This specific SNP was located within an intron in *Alkylated DNA Repair Protein AlkB Homolog 3* (ALKBH3). This gene has been specifically associated with cell survival in various forms of cancer in humans (Tasaki et al., 2011; Stefansson et al., 2017). This SNP was also located in a region described by Peters et al. (2012) that contained several SNP windows associated with various forms of ADG calculated from birth to weaning, weaning to yearling, and birth to yearling in Brangus heifers. In addition to the aforementioned, rs42728110 (PPI, 100%), an additional SNP, rs42491470, was identified on BTA 9 (83% PPI) at 13.8 Mb. Similar to the other SNP identified on BTA 9, no annotated genes were found in this chromosomal region. At 61.2 Mb on BTA 18, rs109374253 was located 13.5 kb from the gene *NLR Family Pyrin Domain Containing 12* (NLRP12) which has been associated with digestive disorders in rabbits (Liu et al., 2013). This SNP is located in a region of the bovine genome that has been associated with still births, calving ease, calf survival, and birth index and net merit in Holstein cattle (Cole et al., 2011; Hoglund et al., 2012). On BTA 22 at 55.1 Mb, SNP rs43598673 (84% PPI) was located within an intron in the gene *ATPase Plasma Membrane Ca2+ Transporting 4* (ATP2B4). This gene has been reported to have a role in calcium regulation of a variety of biological functions ranging from bone formation (Basit et al., 2017) to heart defects such as congenital ventricular arrhythmia (Dewey et al., 2015). At 14.8 Mb on BTA 23, rs109381844 (80% PPI) was found to explain variability in STAY. There were no annotated genes at this location.

Four SNP were identified that were included in the model for STAY at least 75% of the time. Two SNP rs42446897 (75% PPI) and rs42330036 (77% PPI) were identified on BTA 6 at 13.0 and 105.1 Mb, respectively. The SNP rs42446897 was located within an intron in the *Calcium/Calmodulin Dependent Protein Kinase II Delta* (CAMK2D) gene that has been implicated in various roles associated with heart function (Daniels et al., 2015) and lumen formation of mammary tissue (Nguyen and Shively, 2016). Single nucleotide polymorphism rs42446897 (77% PPI) was located in a non-coding region of the *Collapsin Response Mediator Protein 1* (CRMP1) gene whose resulting

protein was found expressed in the nervous system of humans. Lastly, BTA-30939-no-rs (83.5 Mb on BTA 12) was found to be associated with variability in STAY with a 78% PPI. No gene annotation was found for this region of the bovine genome.

Conclusions

These results indicate varying levels of success in identifying QTL associated with HPG and STAY. A lack of data in conjunction with low heritability has limited the identification of any significant QTL for HPG. Given that observations for STAY are formed from weaning records, a greater number of records are typically available as they require no additional data reporting by breeders. Accordingly, a total of 12 SNP were found to be associated with QTL influencing STAY. A number of these are located within previously reported QTL for net merit, ADG, and feed conversion ratio, while others are located within genes mapped throughout the genome. The results herein provide a platform from which to base conclusions from future studies with greater data densities.

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